Α

Primer Extension Coupled with Electrospray Product Ion Mass Spectrometry

PCR product: 3' TTGATCAAGTATAGGGCCGATAA*T*TCGGCATATGCGG...
SNP primer 5' TAGTTCATATCCCGGCTATT

Step 1

50 nM PCR product, 3-4 μM SNP primer

1 μM ddATP, 1 μM ddCTP, 1 μM ddGTP, 1 μM ddTTP

20 mM NH4Ac buffer pH 8.7

2 mM Mg(Ac)₂

1 unit DNA polymerase

15-20 thermal cycles

PCR product: 3' TTGATCAAGTATAGGGCCGATAATTCGGCATATGCGG...
SNP primer 5' TAGTTCATATCCCGGCTATTAdd

Step 2 Reconstitute pass sample remove ma

Reconstituted in 20 mM NH_4Ac buffer and pass sample through a metal chelating resin to remove magnesium from the solution.

Step 3 Analyze the sample solution using Selected Reaction Monitoring
Electrospray Mass Spectrometry to detect the unreacted ddNTPs remaining
in solution relative to a control sample that did not undergo primer extension.

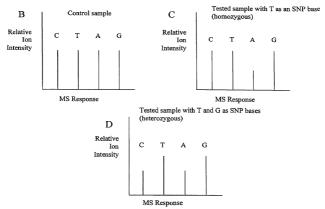


Figure 1

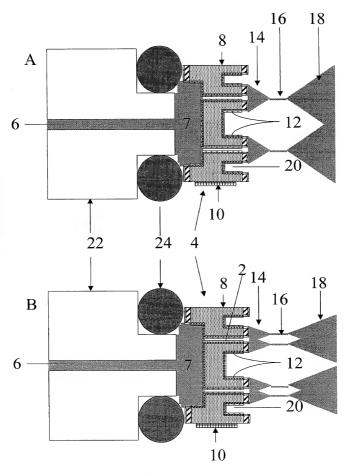


Figure 2

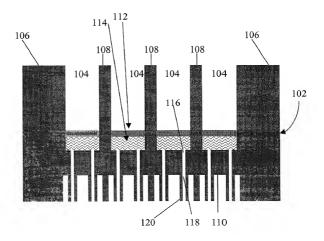


Figure 3A

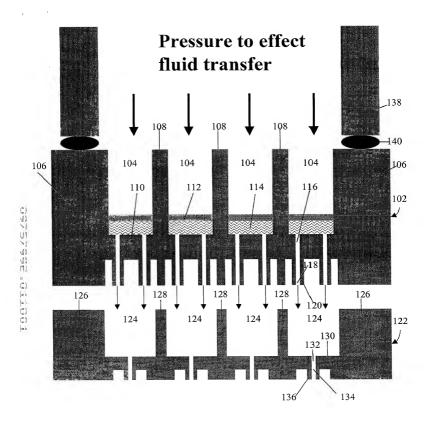


Figure 3B

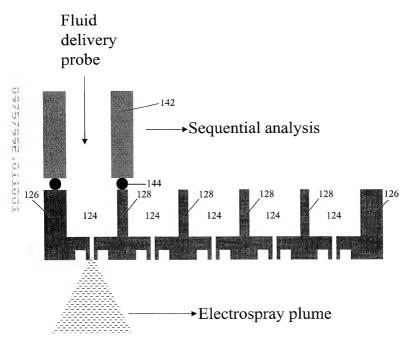


Figure 3C

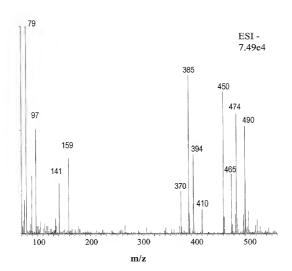


Figure 4

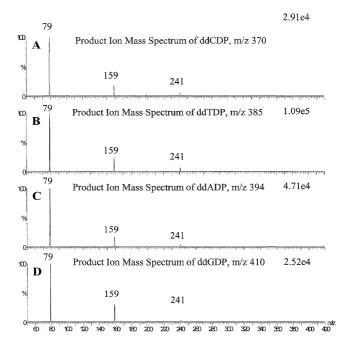
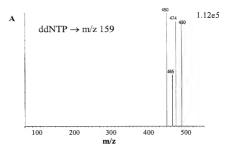


Figure 5



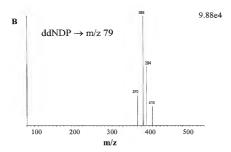


Figure 6

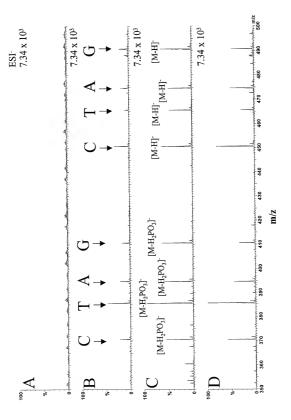


Figure 7

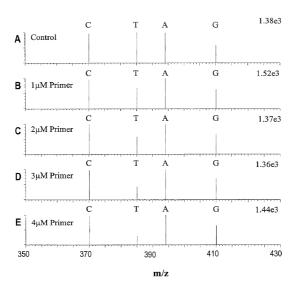


Figure 8

Schematic diagram of SNP genotyping by ESI/MS using synthetic single strand DNA as target templates in homogeneous reaction

Homogeneous templates:

Template A: 5' CCCCTGTATCCTGTGTGAAATTGTTATCCGCTC	3°	(SEQ. ID. No. 1)
SNP primer: 3' AGGACACACTTTAACAATAGGCGA	5'	(SEQ. ID. No. 5)
Decreased ddNTPs: ddTTP (385.1>79)		
Template C: 5' CCCCTGTCTCCTGTGTGAAATTGTTATCCGCTC	3'	(SEQ. ID. No. 2)
SNP primer: 3' AGGACACACTTTAACAATAGGCGA	5'	(SEQ. ID. No. 5)
Decreased ddNTPs: ddGTP (410.1>79)		
Template G: 5' CCCCTGTGTCTGTGAAATTGTTATCCGCTC	3'	(SEQ. ID. No. 3)
SNP primer: 3' AGGACACACTTTAACAATAGGCGA	5'	(SEQ. ID. No. 5)
Decreased ddNTPs: ddCTP (370.1>79)		
Template T: 5' CCCCTGTTTCCTGTGTGAAATTGTTATCCGCTC	3'	(SEQ. ID. No. 4)
SNP primer: 3' AGGACACACTTTAACAATAGGCGA	5'	(SEQ. ID. No. 5)
Decreased ddNTPs: ddATP (394.1>79)	-	(524.15.110.5)

Figure 9

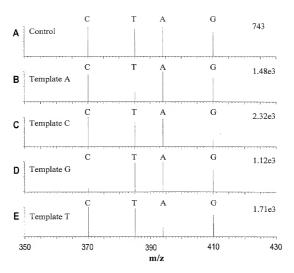


Figure 10

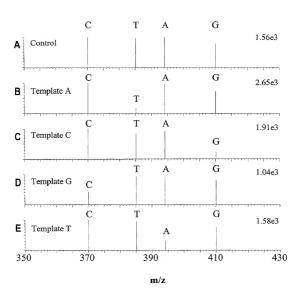


Figure 11

Schematic diagram of SNP genotyping by ESI/MS using synthetic single strand DNA as target templates in heterogeneous reaction

Heterogeneous templates (two templates mixture):

C Template A+C: 5' CCCCTGTATCCTGTGTGAAATTGTTATCCGCTC 3' SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' Decreased ddNTPs: ddTTP (385.1>79) and ddGTP (410.1>79)	(SEQ. ID. No. 2) (SEQ. ID. No. 1) (SEQ. ID. No. 5)
G	(SEQ. ID. No. 3)
Template A+G:5' CCCCTGTATCCTGTGTGAAATTGTTATCCGCTC 3'	(SEQ. ID. No. 1)
SNP primer: 3' AGGACACTTTAACAATAGGCGA 5' Decreased ddNTPs: ddTTP (385.1>79) and ddCTP (370.1>79)	(SEQ. ID. No. 5)
T	(SEQ. ID. No. 4)
Template A+T: 5' CCCCTGTATCCTGTGTGAAATTGTTATCCGCTC 3'	(SEQ. ID. No. 1)
SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' Decreased ddNTPs: ddTTP (385.1>79) and ddATP (394.1>79)	(SEQ. ID. No. 5)
G	(SEQ. ID. No. 3)
Template C+G: 5' CCCCTGTCTCCTGTGTGAAATTGTTATCCGCTC 3'	(SEQ. ID. No. 2)
SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' Decreased ddNTPs: ddGTP (410.1>79) and ddCTP (370.1>79)	(SEQ. ID. No. 5)
T	(SEQ. ID. No. 4)
Template C+T: 5' CCCCTGTCTCCTGTGTGAAATTGTTATCCGCTC 3'	(SEQ. ID. No. 2)
SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' Decreased ddNTPs: ddGTP (410.1>79) and ddATP (394.1>79)	(SEQ. ID. No. 5)
T	(SEQ. ID. No. 4)
Template G+T: 5' CCCCTGTGTCCTGTGTGAAATTGTTATCCGCTC 3'	(SEQ. ID. No. 3)

Figure 12

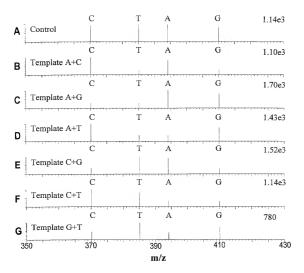


Figure 13

384 bp of partial PheA gene and its primers used for SNP assay:

384bp double-stranded target sequence: (SEQ. ID. No. 6)

5040p double-standed impersoquence. (65-Q. 25.116.0)						
	W338Ind	-		*		
5'			TGTTCTATCT	GGATATTCAG	GCCAATCTTG	AATCAGCGGA
3'	GCCATTAGGT	ACCCTTCTCT	ACAAGATAGA	CCTATAAGTC	CGGTTAGAAC	TTAGTCGCCT
					C374	
					TCAATGAAGG	
	TTACGTTTTT	CGTAACTTTC	TCAATCCCCT	TTAGTGGGCA	AGTTACTTCC	
					•	
	C374Apd -					
					TGAAAAGGTG	
				AGGTTGGACT	ACTTTTCCAC	GGCCTACTAC
	C474S	ou 🗲	V383pu			
					CGCCTGACGC	
	ACTTAGTAGG	CCGTGACCTA	ATAATGACCG	CTAACAGTAA	GCGGACTGCG	TTATTGTGCG
					GAATTCGAGC	
	CCGAAAGTGA	GACTTTTGCG	ACACGCATTA	GCGGCTTGGT	CTTAAGCTCG	AGCCATGGGC
	acar maamam	3 G3 GEGG3 GG	maan aaan ma	ON NOOTHINGO	ACTGGCCGTC	CTTTTT CAAC
					TGACCGGCAG	
	CCCTAGGAGA	TCTCAGCTGG	ACGTCCGTAC	GITCGAACCG	IGACCGGCAG	CAAAAIGIIG
	OTTO TO A CTO	GGAAAACCCT	GGCG 3'			
		CCTTTTGGGA				
	CHITCHLITTAL	- C- L I I I I I I I I I I I I I I I I I I				

Amplification primers:

W338Ip	d 5'-CGGTAATCCAATTGAAGAGATGTTCT-3'	(SEQ. ID. No. 7)
#1224	5'-CGCCAGGGTTTTCCCAGTCACGA-3'	(SEQ. ID. No. 8)

#1224

Polymorphism detection primers:	Polymorphic base
W338Ipd 5'-CGGTAATCCAATTGAAGAGATGTTCT-3'	T (SEQ. ID. No. 7)
C374Spu 5'-TCACTTGGGTAGGATCCCAATACCTTCATT-3'	C (SEQ. ID. No. 9)
#1224 5'-CGCCAGGGTTTTCCCAGTCACGA-3'	G (SEQ. ID. No. 8)
C374Apd 5'-AGGTATTGGGCGCCTACCCAAGTGAG-3'	T (SEQ. ID. No. 10)
T366pd 5'-ACCCGTTCAATGAAGGTATTGGGC-3'	A (SEQ. ID. No. 11)
V383pu 5'-AACAGGCACTACGTTCTCACTTGGGTA-3'	T (SEQ. ID. No. 12)

Figure 14

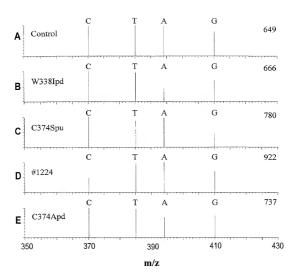


Figure 15

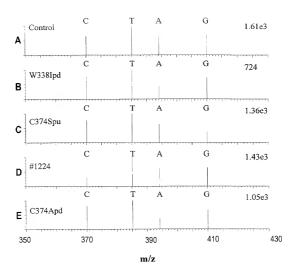


Figure 16

384 bp of partial PheA-C374A mutant gene and its primers used for SNP assay:

384bp double-stranded target sequence: (SEQ. ID. No. 13)

	W338Tpd					
5′	CGGTAATCCA	TGGGAAGAGA	TGTTCTATCT	GGATATTCAG	GCCAATCTTG	AATCAGCGGA
3′	GCCATTAGGT	ACCCTTCTCT	ACAAGATAGA	CCTATAAGTC	CGGTTAGAAC	TTAGTCGCCT

AATGCAAAAA GCATTGAAAG AGTTAGGGGA AATCACCCGT TCAATGAAGG TATTGGGCGC

TTACGTTTTT CGTAACTTTC TCAATCCCCT TTAGTGGGCA AGTTACTTCC ATAACCCGAC

CTACCCAGT GAGAAGGTAG TGCCTGTTGA TCCAACCTGA TGAAAGGTG CCGGATGATG GATGGGTTCA CTCTTGCATC ACGGACAACT AGGTTGGACT ACTTTTCCAC GGCCTACTAC - V383pu

TGAATCATCC GGCACTGGAT TATTACTGGC GATTGTCATT CGCCTGACGC AATAACACGC ACTTAGTAGG CCGTGACCTA ATAATGACCG CTAACAGTAA GCGGACTGCG TTATTGTGCG

GGCTTTCACT CTGAAAACGC TGTGCGTAAT CGCCGAACCA GAATTCGAGC TCGGTACCCG CCGAAAGTGA GACTTTTGCG ACACGCATTA GCGGCTTGGT CTTAAGCTCG AGCCATGGGC

GGGATCCTCT AGAGTCGACC TGCAGGCATG CAAGCTTGGC ACTGGCCGTC GTTTTACAAC CCCTAGGAGA TCTCAGCTGG ACGTCCGTAC GTTCGAACCG TGACCGGCAG CAAAATGTTG

GTCGTGACTG GGAAAACCCT GGCG 3'
CAGCACTGAC CCTTTTGGGA CCGC 5'

W338Ipd 5'-CGGTAATCCAATTGAAGAGATGTTCT-3'

Amplification primers:

V383pu

#1224	5'-CGCCAGGGTTTTCCCAGTCACGA-3'	(SEQ. ID. No. 8)	
Polymorphism	detection primers:	Polymorphic bas	se
W338Ipd	5'-CGGTAATCCAATTGAAGAGATGTTCT-3'	A	(SEQ. ID. No. 7)
#1224	5'-CGCCAGGGTTTTCCCAGTCACGA-3'	C	(SEQ. ID. No. 8)
T366pd	5'-ACCCGTTCAATGAAGGTATTGGGC-3'	С	(SEQ. ID. No. 11)

(SEO. ID. No. 7)

Figure 17

5'-AACAGGCACTACGTTCTCACTTGGGTA-3' C (SEQ. ID. No. 12)

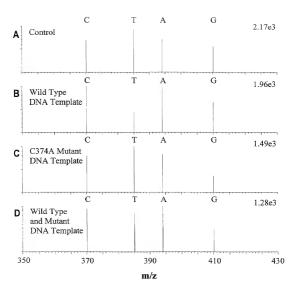


Figure 18

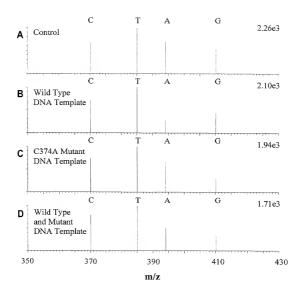
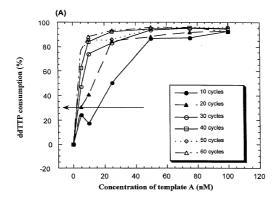


Figure 19



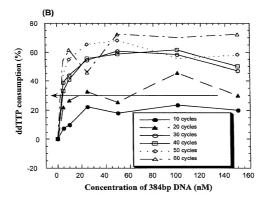


Figure 20